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Mechanisms of cerebrospinal fluid and brain interstitial fluid production

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Abstract

Fluid homeostasis is fundamental for brain function with cerebral edema and hydrocephalus both being major neurological conditions. Fluid movement from blood into brain is one crucial element in cerebral fluid homeostasis. Traditionally it has been thought to occur primarily at the choroid plexus (CP) as cerebrospinal fluid (CSF) secretion due to polarized distribution of ion transporters at the CP epithelium. However, there are currently controversies as to the importance of the CP in fluid secretion, just how fluid transport occurs at that epithelium versus other sites, as well as the direction of fluid flow in the cerebral ventricles. The purpose of this review is to evaluate evidence on the movement of fluid from blood to CSF at the CP and the cerebral vasculature and how this differs from other tissues, e.g., how ion transport at the blood-brain barrier as well as the CP may drive fluid flow. It also addresses recent promising data on two potential targets for modulating CP fluid secretion, the $Na^+/K^+/Cl^-$ cotransporter, NKCC1, and the non-selective cation channel, transient receptor potential vanilloid 4 (TRPV4). Finally, it raises the issue that fluid secretion from blood is not constant, changing with disease and during the day. The apparent importance of NKCC1 phosphorylation and TRPV4 activity at the CP in determining fluid movement suggests that such secretion may also vary over short time frames. Such dynamic changes in CP (and potentially blood-brain barrier) function may contribute to some of the controversies over its role in brain fluid secretion.

Keywords

Blood-brain barrier; Cerebrospinal fluid; Choroid plexus; interstitial fluid; Ion transport; NKCC1; Secretion; TRPV4

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1. Introduction

There are currently some fundamental disagreements with regards to cerebrospinal fluid (CSF) and brain interstitial fluid (ISF) production, movement, and absorption. This review primarily focuses on debates about the former, brain fluid production, and the role of choroid plexus (directions of CSF flow), underlying mechanisms of secretion and the function of specific transporters/ion channels. There are also controversies over fluid movement within the brain that are outside the focus of this paper. These primarily focus on the proposed glymphatic system (Iliff et al., 2012). Readers are referred to excellent recent reviews reflecting on evidence for and against components of that system (Hladky and Barrand, 2022; Rasmussen et al., 2022). Similarly, readers are referred to (Proulx, 2021) on debates on the relative importance of different pathways by which CSF can exit the brain.

These debates partly reflect advances in technology, particularly in imaging (e.g., two-photon and magnetic resonance imaging), that have provided new insights to fluid and solute movement in the brain. However, they also reflect a lack of specific methods to reduce CSF/ISF production and volume in patients. Given the importance of altered brain fluid homeostasis in neurological conditions, there is a dire need to be able to alter fluid balance pharmacologically. Malignant edema after stroke, traumatic brain injury and cerebral neoplasms is life threatening. Hydrocephalus is life altering in both neonates and the elderly (normal pressure hydrocephalus occurs in 5.9% people over 80 (Jaraj et al., 2014)). Current treatments for hydrocephalus are neurosurgically-induced fluid diversion with either a shunt placement or endoscopic third ventriculostomy (ETV) with or without choroid plexus (CP) cauterization (Hochstetler et al., 2022; Warf, 2005), while those for reducing brain edema have not changed substantially for decades and are largely based on osmotic agents and, for brain tumors and bacterial meningitis, corticosteroids (Cook et al., 2020; Zoccarato et al., 2021).

This review gives an overview of the fluid systems within the brain and how they differ from fluid movement in other organs. It then discusses potential sites of fluid production, underlying mechanisms of secretion and evidence that production is not constant. It particularly focuses on recent advances and controversies.

2. Brain fluid systems and comparisons to other tissues

Excluding the vasculature, there are four major brain fluid compartments: CSF within the ventricular system, CSF around the brain in the subarachnoid space (which also communicates with the perivascular space of penetrating arteries and arterioles, as well as venules (Abbott et al., 2018)), ISF within the brain extracellular space (ECS), and intracellular fluid. Communication between ventricular and subarachnoid CSF is via the foramen of Luschka and foramen of Magendie. The traditional view proposed by Cushing, Dandy and Weed over one hundred years ago is that CSF is produced in the ventricles, primarily by the CPs in the lateral, third and fourth ventricles and flows into the subarachnoid space. As described and discussed in the next section, that view has been questioned. Ventricular CSF and brain ISF are in communication across the ventricular wall, while CSF in the subarachnoid space is in communication with ISF across the pial surface

of the brain and it can rapidly enter the perivascular space around penetrating arterioles (one arm of the proposed glymphatic system).

In human, the relative volumes of these compartments are approximately: ventricular CSF ~23 mL; subarachnoid space (including around spinal cord) ~120 mL; brain ISF 250 mL (~20% of brain volume) and intracellular fluid 750 mL (Davson et al., 1987; Nicholson and Hrabetova, 2017). CSF drainage is typically thought to be ~500 mL/day, i.e., CSF turns over ~3–4 times a day. However, there is considerable variation in measured CSF production depending on the methodology used (for recent review of methodologies see (Liu et al., 2022)).

There is also significant blood volume within the brain and changes in blood volume during the cardiac cycle cause oscillations in CSF pressure and CSF redistribution within the CSF system (e.g., through the cerebral aqueduct (Balédent et al., 2001)) and between CSF and ISF through the proposed glymphatic system (e.g., via the perivascular space (Mestre et al., 2018)). The respiratory cycle (thoracic pressure) also causes CSF redistribution (Dreha-Kulaczewski et al., 2017), as do changes in body position (Gergelé and Manet, 2021).

It is important to compare fluid balance in brain with that in other organs. While the size of the brain ECS (~20% of brain volume) is similar to some other tissues (e.g., skeletal muscle (Goodall et al., 2020)), no other tissue has a system analogous to the CSF. In non-nervous system tissues, there is movement of fluid across blood vessels driven by Starling forces, the net balance between hydrostatic and osmotic forces due to plasma proteins (oncotic pressure). The net extravasated fluid, in combination with some water produced by metabolism, results in lymph flow which, through the lymph system, circulates back to the vasculature. Lymph flow clears metabolic waste products from tissues and has an important role in immune surveillance. In humans, whole-body afferent lymph production is about 8–12 L/day (Renkin, 1986). In comparison, brain fluid production (~0.5 L/day) is about 5% of total body lymph production even though the brain is only about 2% of total body weight. That greater fluid production occurs even though cerebral capillaries have hydraulic conductivities (L_ρ) that are orders of magnitude lower than systemic capillaries which would greatly reduce fluid production due to Starling forces. For example, L_ρ is 3.0×10^{-13}, 2.5×10^{-11} and 5.0×10^{-10} $cm^3.s^{-1}.dyne^{-1}$ in brain, skeletal muscle and mesentery, respectively (Renkin, 1992). This reflects the presence of the tight junctions between brain endothelial cells that seal the paracellular space. Oreskovic and Klarica (Oresković and Klarica, 2010) and Oreskovic et al. (Oreskovic et al., 2017) have focused on the importance of hydrostatic and oncotic (Starling) forces in moving fluids into and out of brain across cerebral capillaries. However, the very low L_ρ of cerebral vessels and the relatively greater overall brain fluid production, indicates that there is something fundamentally different between brain fluid production and Starling force-driven fluid production at the vasculature in other organs; e.g., fluid movement across the CP epithelium and/or epithelial-like (ion-driven) fluid secretion across the blood-brain barrier (BBB).

In addition, CSF is not simply a plasma ultrafiltrate (Miner and Reed, 1972). Apart from very low protein contents, CSF and ISF have differences in ion composition from plasma including a lower K^+ concentration and the CSF and ISF concentrations of some ions (e.g.,

K^+ and Ca^{2+}) are very tightly regulated during changes in plasma composition (e.g., (Jones and Keep, 1987; Jones and Keep, 1988)). This is important for preventing changes in plasma composition from impacting neuronal activity and it may underlie the difference between the mechanisms of brain and systemic tissue fluid production.

3. The choroid plexus as the site of CSF secretion

3.1. Choroid plexus morphology and polarization

The CPs in the lateral, 3rd and 4th ventricles are highly vascularized. They have a single layer of cuboidal epithelial cells surrounding fenestrated capillaries that are separated by connective tissue with stromal cells (Fig. 1). The epithelial cells have an apical (CSF-facing) brush border, basolateral interdigitations, apically located tight junctions, and a high mitochondrial content typical of a secretory epithelium. Unlike in brain parenchyma, where there is a BBB at the level of the cerebral endothelium and their tight junctions, it is the CP epithelial cells and their tight junctions that form a blood-CSF barrier. The CP endothelium is fenestrated which facilitates water movement.

As well as being structurally polarized, there are marked differences in ion transporter and ion channel distribution between the apical and basolateral membranes. In addition, the water channel, aquaporin (AQP) 1 is preferentially expressed on the apical membrane (Fig. 2; (Praetorius and Damkier, 2017)). This ion transporter/channel polarization has long been thought to be important in CSF secretion and CP ion transporter inhibitors can reduce CSF secretion in animals (Praetorius and Damkier, 2017). However, except carbonic anhydrase inhibition (acetazolamide), which affects the activity of multiple HCO_3^- and H^+ transporters, those results have not yet translated to the clinic suggesting a greater understanding of CSF secretion is needed.

3.2. Evidence that the choroid plexus does or does not secrete CSF

At a time when the multiple roles of the CP in brain physiology and pathophysiology are becoming better understood (Kratzer et al., 2020; Solár et al., 2020), the role of the CP in CSF and overall brain fluid secretion is being questioned. The traditional view is that the CP accounts for the majority of CSF production (Spector et al., 2015a; Spector et al., 2015b), but it has been proposed that the CP does not secrete CSF (Oresković and Klarica, 2010; Oreskovic et al., 2017) or even that it can clear CSF (Sadegh et al., 2023; Xu et al., 2021). Evidence for these different points of view is briefly discussed here (and Table 1).

Several very different in vitro models have shown the CP epithelium can secrete fluid apically. Those models include porcine primary cultures, a human papilloma cell line and human CP organoids (Hakvoort et al., 1998a; Hakvoort et al., 1998b; Hulme et al., 2022; Pellegrini et al., 2020). As always, there are concerns over whether these models reflect the in vivo condition. An intermediate model to fully in vivo measurements, is the in situ perfused cat and sheep choroid plexus where fluid secretion is found and can be sampled (e.g., (Chen et al., 2009; Miner and Reed, 1972)). In vivo, there is evidence that the CP is a major site of blood to brain Na^+ and Cl^- transport (the primary solutes in CSF) (reviewed in (Spector et al., 2015a)); ion transport inhibitors that target transporters present at the

CP reduce CSF production in animals (e.g., (Javaheri and Wagner, 1993; Steffensen et al., 2018), see also reviews (Praetorius and Damkier, 2017; Spector et al., 2015a)); genetic deletion of CP ion transporters and the CP water channel, aquaporin 1, alter ventricle size and CSF secretion (Jacobs et al., 2008; Kao et al., 2011; Oshio et al., 2005); blood hematocrit is greater in choroidal venous blood compared to arterial blood (Welch, 1963); blocking outflow from the ventricular system can cause ventriculomegaly (McAllister 2nd et al., 1991; Williams et al., 2014), and lateral ventricle CP extirpation reduces CSF flow and Na^+ transport 25–40% in rhesus monkeys ((Milhorat et al., 1976; Milhorat et al., 1971); please note that the 4th ventricle CP has approximately equal weight to the two lateral ventricle CPs). In addition, clinical studies have reported that combining ETV with bilateral CP cauterization improves outcomes compared to ETV alone (Stone and Warf, 2014; Warf, 2005; Warf et al., 2012) and it is the subject of a current clinical trial in North America (NCT04177914).

However, the role of the CPs in CSF secretion has been doubted (e.g., (Brinker et al., 2014; Oresković and Klarica, 2010; Oreskovic et al., 2017)) with proposals that brain fluid is produced and absorbed at the cerebrovasculature (see comments in sections 2 and 4). Evidence against the CP-mediated CSF production includes a lack of a CP-generated hydrostatic gradient, clinical cases where obstruction of the cerebral aqueduct has not resulted in hydrocephalus, animal experiments where the lateral/3rd ventricles are isolated by occlusion or cannulation of the cerebral aqueduct without ventricular dilation or evidence of CSF production, species where there is a separation between ventricular CSF and CSF surrounding the brain (sharks), and human studies where CP cauterization had no effect of hydrocephalus development (Oreskovic et al., 2017). Much has also been made of the rapid influx of isotopic water across the cerebrovasculature (Oreskovic et al., 2017), an alternate potential site of fluid influx to the CP, but influx of isotopic water does not necessarily reflect net fluid flow. The latter depends on the difference between influx and efflux and the latter is also very rapid (Hladky and Barrand, 2016).

In humans, a number of magnetic resonance imaging (MRI) techniques have been developed to assess CSF movement in patients, such as using cardiac-gated phase contrast MRI to examine net CSF flow through the cerebral aqueduct (Liu et al., 2022; Mehta et al., 2022). In general, those methods have found a net movement of CSF from the ventricular system (anterograde), although there is considerable variation in calculated CSF flow across methods (reviewed in (Liu et al., 2022)). However, determining net flow through the cerebral aqueduct is complicated by the magnitude of the bidirectional movement of CSF during the cardiac and respiratory cycles. For example, Eisma et al. (Eisma et al., 2023) reported a net (caudal) flow of CSF of 0.245 mL/min in patients but an absolute flow (summation of bidirectional flow) of 4.86 mL/min, i.e., 87% of CSF was regurgitated (retrograde flow) back into the ventricles during the cardiac cycle. Measuring CSF flow at the aqueduct during the cardiac cycle is also confounded by the impact of the respiratory cycle (Spijkerman et al., 2019).

Not all human MRI studies, however, have found a net caudal CSF movement. For example, Eide et al. (Eide et al., 2021) found evidence of net retrograde CSF flow through the aqueduct from the 4th to the 3rd ventricle in patients with communicating

hydrocephalus and Kim et al. (Kim et al., 1999) also reported retrograde flow in patients with normal pressure hydrocephalus. Bateman and Brown (Bateman and Brown, 2012) reported retrograde flow though the aqueduct in children under 2 years old but anterograde flow at older ages.

Evidence that the CP can act to clear CSF has also been generated by Xu et al. (Xu et al., 2021) and Sadegh et al. (Sadegh et al., 2023) in mice. That group overexpressed the $Na^+/K^+/Cl^-$ cotransporter, NKCC1, at the CP and found it reduced ventriculomegaly in a neonatal mouse model of obstructive hydrocephalus (Xu et al., 2021) and in fetal, neonatal and adult mouse models of post-hemorrhagic hydrocephalus (Sadegh et al., 2023). While bumetanide-sensitive $Na^+/K^+/Cl^-$ cotransport has been implicated as being involved in CSF secretion in dogs and rodents (Javaheri and Wagner, 1993; Karimy et al., 2017; Steffensen et al., 2018), that transporter is bidirectional dependent upon prevailing ion gradients. Xu et al. (Xu et al., 2021) reported very high CSF K^+ concentrations in the neonatal mouse that might drive net $Na^+/K^+/Cl^-$ cotransport and fluid transport from CSF to blood and Sadegh et al. (Sadegh et al., 2023) propose that K^+ release from red blood cells after intraventricular hemorrhage increases in CSF K^+ concentration.

One possible explanation for these discrepant findings on the role of the CP in brain fluid secretion might be if net fluid flow across the CP epithelium is much more labile (changing with time and physiological/pathophysiological state) than generally thought. This is discussed further in section 6.

3.3. Mechanisms underlying choroid plexus fluid secretion

Three mechanisms or combination of mechanisms that may underly CP CSF secretion are: vectorial ion transport produces an osmotic gradient that draws water across the epithelium; a hydrostatic pressure gradient between blood and CSF may drive fluid paracellularly through the epithelial tight junctions; specific transporters may co-transport water with solutes (Fig. 3).

The traditional model is that the transport of ions across the CP produces osmotic gradients that entrain water movement across the epithelium. However, there is only a small osmotic gradient between CSF and blood and modeling suggests this is insufficient to drive CSF secretion (Razzaghi Khamesi et al., 2023). In addition, secretion continues even when plasma osmolality is greater than CSF (Oernbo et al., 2022). It is, however, possible there might be local gradients in the spaces between the epithelial apical microvilli or within the basolateral interdigitations but those are difficult to access experimentally.

Water movement may follow ion transport via AQP water channels. The CP epithelium expresses AQP1 on the apical membrane and AQP1 null mice have a ~ 25% reduction in CSF secretion (Oshio et al., 2005). Other aquaporins have been identified at the choroid plexus, but their physiological importance is unclear (Municio et al., 2023).

While the CP epithelial cells are linked by tight junctions, hydrostatically driven paracellular fluid flow may contribute to CSF secretion. In contrast to the BBB, the CP is normally considered a leaky barrier with a relatively low electrical resistance across the epithelium

(Zeuthen, 1991), although in vitro a porcine CP epithelial cells had transepithelial electrical resistances of ~1700 $\Omega.cm^2$ (Hakvoort et al., 1998a). In barrier tissues, transmembrane tight junction proteins (claudins, occludin) occlude the paracellular space, but certain claudins form pores allowing ion and water movement (Krug et al., 2012). Thus, while claudin-5, the predominant claudin at the BBB, does not form pores, the CP epithelium expresses a range of claudins (−1, −2, −3 and − 11, (Kratzer et al., 2012; Steinemann et al., 2016; Wolburg et al., 2001)) and at least one of those, claudin-2, does form water channels (Rosenthal et al., 2010). While claudin-2 deficient mice are outwardly normal (Muto et al., 2010), the effects of claudin-2 loss on CSF dynamics (and potential compensatory mechanisms) merits investigation. While CSF is not a plasma ultrafiltrate, it is possible that some fluid traverses the epithelium via the paracellular route and its composition is modified by epithelial transporters.

Another possibility is that some CP epithelium transporters may directly transport water. Thus, for example, there is evidence that the $Na^+/K^+/Cl^-$ co-transporter, NKCC1, can transport H_2O as well as ions. The readers are referred to MacAulay et al. (MacAulay et al., 2022) for evidence for this type of transport. It should be noted that NKCC1 appears to transport ~590 molecules of water per cycle (MacAulay and Zeuthen, 2010) making the fluid secretion almost isotonic to plasma and CSF.

One problem with elucidating which potential mechanisms are involved in CP fluid transport has been a paucity of in vitro models demonstrating fluid secretion. In a series of papers, Galla and colleagues examined primary porcine CP epithelial cells cultured on a transwell under serum free conditions. They found monolayers with very high transepithelial electrical resistances (>1500 $\Omega.cm^2$) that could secrete fluid (Hakvoort et al., 1998a; Hakvoort et al., 1998b; Haselbach et al., 2001). They were able to show a role of Na/K-ATPase in that secretion as well as a dependence on $NaHCO_3$. Very recently, Hulme et al. (Hulme et al., 2022) have examined a human choroid plexus papilloma cell line (HIBCPP) with transepithelial electrical resistances of >400 $\Omega.cm^2$. They found a marked increase in fluid secretion with a transient receptor potential vanilloid 4 (TRPV4) channel agonist, GSK1016790A (Hulme et al., 2022). Recently, Pellegrini et al. (Pellegrini et al., 2020) have found that human CP organoids can secrete a CSF-like fluid. Importantly, transcriptomics and proteomics showed a high degree of similarity between those organoids and the CP in vivo. Overall, there is the need for more in vitro elucidation of the mechanisms involved in CSF secretion using these new models as well as co-cultures of CP epithelial cells with ‘perfused’ endothelial cells and potentially the generation of vascularized CP organoids. Vascularized brain organoids are currently being generated to examine neurovascular unit interactions (Sun et al., 2022). In addition, it would be informative to examine whether the CP epithelium can be driven to absorb CSF. For example, would high apical K^+ concentrations cause apical to basal fluid reabsorption by altering the ion concentration gradients that have been proposed to drive NKCC1-mediated fluid movement as suggested by the work of (Sadegh et al., 2023; Xu et al., 2021)?

3.4. Role of specific choroid plexus transport mechanisms

Recently, there has been great interest in the roles of the $Na^+/K^+/Cl^-$-co-transporter, NKCC1 (Slc12a2), and a Ca^{2+}-permeable, non-selective cation channel, transient receptor potential vanilloid 4, TRPV4, in regulating CSF secretion. NKCC1 is highly expressed on the apical membrane of the CP epithelium in rodents and humans (Plotkin et al., 1997; Praetorius and Nielsen, 2006). It is inhibited by bumetanide, a loop diuretic with poor brain penetration. Thus, Steffensen et al. (Steffensen et al., 2018) used intracerebroventricular (icv) infusion of bumetanide to access the apical NKCC1 and found a ~ 50% reduction in CSF secretion rate in mice with the inhibitor. This degree of CSF production inhibition is similar to older results from Javaheri and Wagner (Javaheri and Wagner, 1993) in dogs with icv bumetanide. In addition, Karimy et al. (Karimy et al., 2017) found a 3-fold stimulation of bumetanide-inhibitable CSF secretion after intraventricular hemorrhage (IVH) in rats due to NKCC1 phosphorylation and simulation and that it contributes to post-hemorrhagic hydrocephalus. Metayer et al. (Metayer et al., 2022) also found that icv bumetanide reduced acute hydrocephalus in a rat model of subarachnoid hemorrhage.

There are efforts to produce more brain permeable NKCC1 inhibitors (Loscher and Kaila, 2022), but another approach is to target regulators of NKCC1 activity. Thus, Karimy et al. (Karimy et al., 2017) found that IVH-induced stimulation of bumetanide-inhibitable CSF secretion was an inflammatory response involving toll-like receptor-4 (TLR4) activating STE20/SPS1-related, proline-alanine-rich kinase (SPAK) which in turn phosphorylates and activates NKCC1.Targeting TLR4 or SPAK normalized CSF secretion. Robert et al. (Robert et al., 2023) have found that the same TLR4-SPAK-NKCC1 pathway causes CSF hypersecretion after bacterial infection and targeting that pathway normalizes secretion. Zhang et al. (Zhang et al., 2020) have recently developed a novel SPAK inhibitor, ZT-1a that inhibits NKCC1 by decreasing SPAK-dependent phosphorylation and reduces IVH-induced CSF hypersecretion. Zhang et al. (Zhang et al., 2022) have targeted the NLRP3 inflammasome using an inhibitor or a genetic knockout to reduce NKCC1 phosphorylation and CSF hypersecretion in a rat IVH model. Toft-Bertelsen et al. (Toft-Bertelsen et al., 2022) have also found that IVH increases lysophophatidic acid (LPA) concentrations in CSF and that icv LPA can enhance NKCC1 and CSF secretion by activating TRPV4. Xu et al. (Xu et al., 2022) found a loss of protein tyrosine phosphatase non-receptor type 20 (Ptpn20) in the H-Tx rat model of hydrocephalus. They then determined that Ptpn20 deletion in mice causes increased phosphorylation of NKCC1 and ventriculomegaly from week eight indicating Ptpn20 as an important NKCC1 regulator.

However, there are inconsistencies in the literature on the effects of targeting NKCC1. Vogh et al. (Vogh and Langham Jr., 1981) and Bothwell et al. (Bothwell et al., 2021) in rats found no change in CSF production after systemic bumetanide delivery. This might be due to the poor brain penetration of bumetanide. However, in in vitro studies on single CP epithelial cells, Gregoriades et al. (Gregoriades et al., 2019) found evidence of NKCC1 acting in the inward direction using genetic and pharmacological approaches. They propose that NKCC1 is involved in regulating CP epithelial cell volume and intracellular Cl^- concentration rather than being directly involved in CSF secretion. Evidence for inwardly directed NKCC1-mediated transport and CSF clearance has also been described by Xu et al. (Xu et al., 2021)

and Sadegh et al. (Sadegh et al., 2023). Xu et al. (Xu et al., 2021) found that overexpression of NKCC1 at the CP increased clearance of K^+ from CSF, reduced ventricular volume and, importantly, decreased ventriculomegaly in a neonatal mouse model of obstructive hydrocephalus. Similarly, Sadegh et al. (Sadegh et al., 2023) found overexpression also reduced ventriculomegaly in fetal, neonatal and adult mouse models of post-hemorrhagic hydrocephalus.

One possible reason for this inconsistency is that NKCC1 can act as a net inward or outward transporter depending on the concentration gradients for Na^+, K^+ and Cl^- (highlighted in (Alvarez-Leefmans, 2020; MacAulay and Rose, 2020)). The Xu et al. (Xu et al., 2021) study reported high CSF K^+ concentrations in fetal and neonatal mice (8–10 mM vs. 3 mM in adult) and the Sadegh et al. (Sadegh et al., 2023) study posited that NKCC1-mediated transport may be driven in the CSF to CP direction by K^+ release from the hematomal red blood cells. At the CP, the epithelial concentrations may depend upon the physiological state of the animal (e.g., H^+ and HCO_3^- concentrations) as the cells express Na^+/HCO_3^- co-transporters (NBCn1 and NBCe2), a Cl^-/HCO_3^- exchanger (AE2), a $Na^+/Cl^-/HCO_3^-$ transporter (NCBE) and a Na^+/H^+ exchanger (NHE1) (Fig. 2). Thus, alkalosis or acidosis might impact NKCC1 activity and potentially direction. Inhibition of carbonic anhydrase that catalyzes the conversion of CO_2 and water to H^+ and HCO_3^- reduces CSF production. It is, therefore, extremely important to monitor the physiological state of animals during experiments and, where possible, perform experiments in the conscious state.

In their studies, Xu et al. (Xu et al., 2021) and Sadegh et al. (Sadegh et al., 2023) used icv injection of adeno-associated virus (AAV)2/5 to overexpress NKCC1 at the CP epithelium. AAV2/5 has tropism for the CP. Genetic manipulations specifically at the CP should be very useful for determining the role of specific ion transporters and ion channels at the CP. Jang & Lehtinen (Jang and Lehtinen, 2022) have recently described advances in that field.

TRPV4 is a Ca^{2+}-permeable non-selective cation channel that is osmo- and pressure-sensitive. It is found on the apical membrane of the CP epithelium (Liedtke et al., 2000; Preston et al., 2018). TRPV4 has been implicated in regulating CSF secretion. In a human choroid plexus papilloma cells line (HICPP), Hulme et al. (Hulme et al., 2022) found that a TRPV4 agonist caused a marked increase in CSF secretion. Very importantly, Hochstetler (Hochstetler et al., 2020) found that two TRPV4 antagonists could reduce ventriculomegaly in a rat model of hydrocephalus.

Multiple effects of TRPV4 on CP epithelial ion fluxes have been described. Takayama et al. (Takayama et al., 2014) found that TRPV4 regulates chloride channels. Preston et al. (Preston et al., 2018) found that TRPV4 activation increases transepithelial ion flux and conductance via a mechanism involving a Ca^{2+-} activated K^+ channel (KCNN4c). In HICPP cells, Hulme et al. (Hulme et al., 2022) found that TRPV4 activation alters ion fluxes and increases conductance changes associated with increased fluid secretion. They described involvement of a number of different pathways including protein kinase C, phospholipase C and phosphoinositide 3-kinase in those changes. Toft-Bertelsen et al. (Toft-Bertelsen et al., 2022) found that lysophophatidic acid-induced NKCC1 activation and CSF hypersecretion

via activation of TRPV4. Overall, these results indicate that TRPV4 may have a pivotal role in controlling ion and water flux at the CP epithelium.

In contrast to the above results, Bothwell et al. (Bothwell et al., 2021) found no decrease in CSF secretion rate in rats given 10 mg/kg of a TRPV4 antagonist, RN1734, intraperitoneally. Whether this reflects drug concentrations at the choroid plexus apical membrane is uncertain. Developing methods of targeting TRPV4 to regulate brain water homeostasis are warranted.

While this section has focused on new information on the role of NKCC1 and TRPV4 in CP fluid secretion, there are multiple potential ion transporter/channel targets that need further exploration (Fig. 2; (Praetorius and Damkier, 2017)). Similarly, much work is needed to discover the pathways involved in regulating CP ion transport/fluid secretion. For example, Zeng et al. (Zeng, 2023) recently found that activation of retinoic acid receptor α (RARα) could reduce the CSF hypersecretion and hydrocephalus that occurs in spontaneous hypertensive rats (SHRs) during development. RARα may modulate the inflammatory response in those animals but whether it modulates ion transport is unknown.

4. The cerebral capillaries as the site of ISF/CSF secretion

Cerebral endothelial cells and their linking tight junctions are the primary site of the BBB, although barrier function is regulated by the other cells and extracellular matrix of the neurovascular unit, including pericytes and astrocytes (Fig. 4). Betz and Goldstein (Betz and Goldstein, 1978) first demonstrated that cerebral endothelial cells have epithelial-like properties with a polarized distribution of amino acid transporters. They also found that Na^+/K^+-ATPase is present on the abluminal membrane (Betz et al., 1980). Such polarized ion transporter distribution (Fig. 5) might form the basis for active fluid secretion across the BBB, as is the case for epithelia. There is movement of Na^+ and Cl^- (the primary components of ISF as well as CSF) from blood to brain across the BBB and the influx rate constants for the two ions, ~2 μl/g brain/min in the rat (Betz et al., 1994), could account for all brain fluid production. There is also evidence that blood to brain Na^+ movement is carrier and ion channel mediated (Betz, 1983; Chen et al., 2015; Ennis et al., 1996; O'Donnell, 2014). There are, however, two points to address. The first is that the transfer coefficients (s^{-1}) for Na^+ and Cl^- transport from blood to CSF across the choroid plexus are 20 to 30-fold greater than for blood to brain across the BBB (Smith and Rapoport, 1986). The second is that while brain influx of Na^+ and Cl^- (and other ions) across the BBB has been determined, it is much more difficult to assess the rate of efflux in vivo and it is net ion movement that will determine water movement.

In epithelial tissues, in vitro preparations have been a great asset for examining fluid movement. Unfortunately, most in vitro models using cultured cerebral endothelial cells have had low transendothelial electrical resistances compared to in vivo and have demonstrated transporter down regulation limiting their use for ion transporter studies. Newer human induced pluripotent stem cell (iPSC) derived brain endothelial-like cells do demonstrate transendothelial electrical resistances similar to that in vivo, but the relative protein expression of ion transporters in those preparations is still unknown. Another

potential approach to specifically examine the role of brain endothelial cell ion transport is to specifically knockout/knockdown transporters in those cells. Global knockouts have the disadvantage of impacting other tissues (particularly the kidney) complicating data interpretation. Similarly, 'endothelial-specific' knockouts (e.g., using the Tie2 promoter) may have effects on the systemic as well as the brain vasculature. Using 'brain endothelial cell-specific' approaches (e.g., using the claudin-5 promoter) might be very useful in determining the role of BBB ion transport in brain fluid balance.

As noted above, the paracellular route has a role in fluid transport in specific epithelia where specific claudins can form ion/water pores (Krug et al., 2012; Rosenthal et al., 2010). However, at the cerebral endothelium claudin-5 predominates (Greene et al., 2019) as reflected by a very high transendothelial electrical resistance (Butt et al., 1990). Another potential route of fluid movement is transcytosis but cerebral capillaries have relatively few vesicles compared to systemic capillaries (Stewart, 2000).

Could there be specialized properties of the cerebral arteries/arterioles and veins/venules compared to capillaries that might significantly contribute to brain to water flux, even though the area of such vessels is much less than that of the brain capillary bed? There are differences in gene expression between arterial, capillary and venous brain endothelial cells (zonation (Vanlandewijck et al., 2018)) that may cause differences in function. However, it should be noted that measurements of the transendothelial electrical resistance of arterial and venous pial vessels were very high (~1500 and 900 $\Omega.cm^2$) (Butt et al., 1990) similar to that calculated for the brain parenchyma and claudin-5 mRNA levels are very high in arteriole, capillary and venule endothelial cells (Vanlandewijck et al., 2018). Nevertheless, most of our functional information on blood to brain transport at the cerebrovasculature concerns capillaries, with a relative dearth of data on larger caliber vessels, and their function merits more study.

Overall, it appears likely that the cerebral capillary endothelial cells are the major source of ISF production outside the choroid plexus. However, more work is urgently needed to determine the underlying mechanisms and potential differences along the vascular tree.

5. Metabolic water

Metabolism is an often-neglected source of brain fluid production. The primary energy source of the brain is glucose and the oxidative metabolism of one mole of glucose produces six moles of water and CO_2. Thus, it has been estimated that this results in approximately 60 ml of water per day (Hladky and Barrand, 2016). However, it should be noted that without an influx of solutes from blood, the metabolic water could diffuse down an osmotic gradient into blood.

6. Variations in brain fluid secretion

Both the blood-CSF barrier at the CP and the BBB at the cerebral endothelium are dynamic interfaces between blood and brain rather than 'static walls'. Thus, for example, CSF production is not constant with a marked circadian rhythm in CSF secretion. In human, Nilsson et al. (Nilsson et al., 1992) reported maximum CSF secretion rate of 42 mL/h at

02:00 h and a nadir of 12 mL/h at 18:00 h (a ~ 70% decrease) and there is a need for studies with current MRI technologies to assess CSF secretion during the night. In patients with hydrocephalus, Stephensen et al. (Stephensen et al., 2006) also reported higher intracranial pressures at night. The CP has its own circadian rhythm (Christensen et al., 2022; Myung et al., 2018) and it can influence the 'central' suprachiasmatic nucleus circadian rhythm via the CSF (Myung et al., 2018).

Whether there is also regulation of CP function and CSF secretion over shorter time frames (minute to minute) to meet changes in brain function deserves further investigation. The importance of phosphorylation in regulating NKCC1 function and the evidence that activity of an ion channel, TRPV4, regulates CSF secretion supports that concept. If the rate of CP CSF secretion is very labile, it may contribute to some of the controversies in the literature on the role of the CP in CSF production and raise issues as to how best to assess brain fluid secretion. It also raises the question of what types of signals may regulate fluid movement at the CP? Current evidence is on changes in CSF composition being a signal. Thus, IVH and the release of hemoglobin into CSF trigger inflammatory signaling at the CP increasing CSF secretion (Karimy et al., 2017; Robert et al., 2023) and changes in CSF K^+ concentration also impact CP fluid movement/direction (Sadegh et al., 2023; Xu et al., 2021). Such signaling and alternative regulatory pathways merit further investigation.

Whether there might also be temporal (circadian or acute) variations in BBB fluid secretion merits investigation. It should be noted that $Na^+/K^+/Cl^-$ cotransport is proposed to be involved in fluid transport at the cerebral endothelium (O'Donnell, 2014) suggesting there may well be.

Changes in CSF secretion have been reported with aging. For example, Chen et al. (Chen et al., 2009) reported a ~ 53% decline in CP CSF secretion in sheep between 1 and 2 and 7+ years old. Preston (Preston, 2001) reported a similar ~46% decline in CSF secretion between 3 and 30 months of age in the rat. Also in the rat, Chiu et al. (Chiu et al., 2012) found an initial increase in CSF production between 3 and 12 months but then a 30% decrease by 30 months. Changes in CSF secretion with age occur in the setting of increased ventricular and total CSF volume leading to reduced CSF turnover with implications for waste clearance from brain (Chiu et al., 2012).

In humans, results related to the impact of age on CSF secretion are mixed. For example, May et al. (May et al., 1990) found decreased CSF secretion and Eisma et al. (Eisma et al., 2023) found decreased net caudal CSF flow at the aqueduct of Sylvius with aging (although the latter study also found total (bidirectional) CSF flow at the aqueduct increased with age). In contrast, Gideon et al. (Gideon et al., 1994) found no change in net flow and Sartoretti et al. (Sartoretti et al., 2019) found an increase in net caudal CSF flow at the aqueduct of Sylvius with aging. The reasons for such differences are still uncertain. It should be noted, however, that there is a very marked increase in ventricular and total cranial CSF volume with age (Alisch et al., 2021; Matsumae et al., 1996) meaning that even if CSF secretion remains constant with age, the rate of CSF turnover would decrease.

Changes in CSF secretion rate with age are associated with altered CP epithelial structure/function. Thus, the CP epithelium becomes flattened with a thicker basement membrane during aging in human and rodents (Preston, 2001; Scarpetta et al., 2023b; Serot et al., 2000). Serot et al. (Serot et al., 2000) found the effect of aging on epithelial flattening was exacerbated with Alzheimer's disease. Scarpetta et al. (Scarpetta et al., 2023a) have also recently reported reductions in microvilli height and reduced mitochondria density with aging in mice. Many CP ion transporters are energy dependent implying that the lower mitochondrial content may reduce ion transport and, thus, CSF secretion. Indeed, Chen et al. (Chen et al., 2009) found reduced CP Na^+ transport in aged sheep compared to young animals. The impact on fluid secretion of age-related increased CP epithelial and cerebral endothelial (Banks et al., 2021) basement membrane thickness is unclear. However, there is growing evidence on the impact of integrin-mediated extracellular matrix/cell signaling on cell function, particularly at the cerebral endothelium and the BBB (Halder et al., 2022) which may be impacted by age and basement membrane remodeling.

Changes in CSF/ISF secretion also occur in disease states. While marked increases in CSF secretion in CP papillomas reflect increased tissue size (Fujimura et al., 2004; Milhorat et al., 1976), changes in other diseases reflect changes in ion transport. Thus, as noted above, IVH and CSF bacterial infection can induce CSF hypersecretion via NKCC1 phosphorylation (Karimy et al., 2017; Robert et al., 2023). There is also evidence that ischemic stroke alters ion transport at the BBB thereby inducing edema formation. For example, O'Donnell (O'Donnell, 2014) has found that ischemia enhances Na^+/H^+-antiporter and $Na^+/K^+/Cl^-$ cotransporter activity at the brain endothelial cell luminal membrane resulting in hypersecretion of Na^+, Cl^- and water. Another change that occurs at the brain endothelium and other cells of the neurovascular unit is the new expression of a non-selective cation channel, sulfonylurea receptor-1 (SUR1)-transient receptor potential cation channel subfamily M member 4 (TRPM4) (Simard et al., 2006; Woo et al., 2020). Considerable evidence indicates a role of SUR1-TRPM4 in brain edema formation (Jha et al., 2021). It can be inhibited by glibenclamide (glyburide) and that drug is undergoing clinical trials for stroke and traumatic brain injury (including a Phase 3 trial for large hemispheric infarction; NCT02864953).

The extent to which changes in CSF secretion occur in variety of human diseases is a matter for debate and needs to be a major focus for further research using a range of methodologies. Thus, for example, Eide et al. (Eide et al., 2021) found that aqueductal flow reverses in communicating hydrocephalus, while Tariq et al. (Tariq et al., 2022) reported that some patients with intracerebral and subarachnoid patients have very marked CSF hypersecretion (6 or 7-fold) while others have normal CSF production. Interestingly, they found the former did better clinically.

7. Conclusions

Identifying methods for ensuring brain fluid homeostasis is a very major medical need. Controversies in the field abound, but a greater understanding of the mechanisms of CSF/ISF secretion (as well as clearance from the brain) should aid in developing new therapeutic approaches. Advances in imaging (e.g., two-photon and MRI) have provided

important new insights into fluid movement within and out of the brain, including in human. However, examining movement into brain poses unique challenges including determining regional net (as compared to overall) water and ion movement from blood to brain. This limits our ability to assess the role of BBB fluid transport and how to manipulate it. It is hoped that new advances in in vitro CP and BBB models, including the use of human iPSCs, will provide greater insights into how to manipulate fluid secretion but there will need to be strict benchmarking to ensure that results reflect what occurs in vivo. In addition, translation of such results may be complicated by effects on other tissues (e.g., kidney). An important issue that needs to be addressed is whether CSF/ISF secretion varies over acute time frames (e.g., minute to minute) and the underlying regulatory mechanisms. This may give new approaches for regulating brain fluid balance and perhaps provide insight into some of the controversies concerning CSF/ISF secretion.

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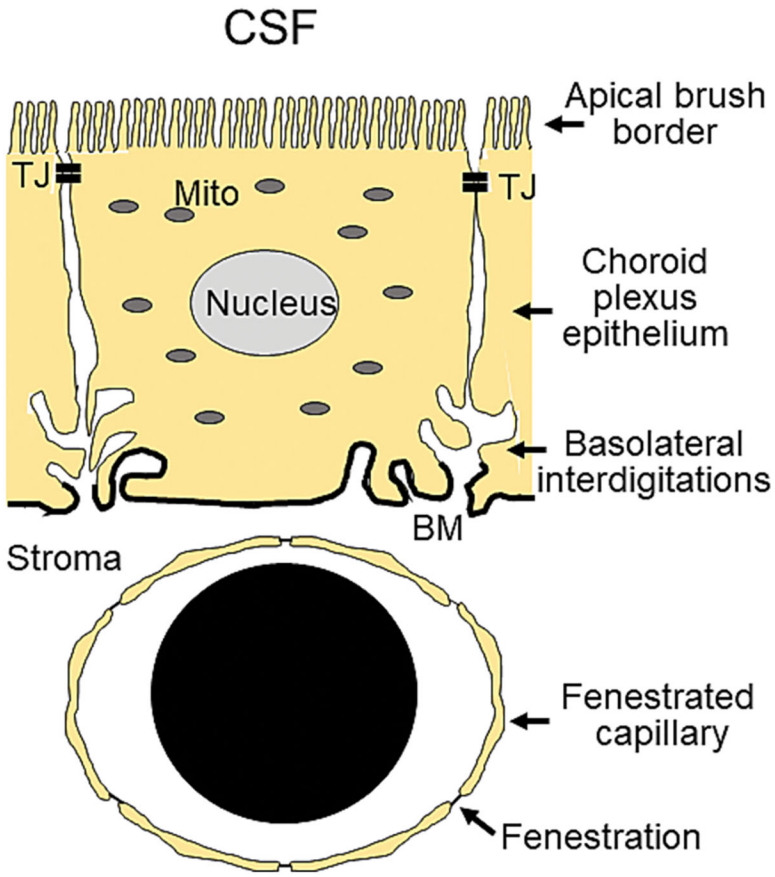

Fig. 1.

General morphology of the choroid plexus epithelium. The epithelium receives blood supply from fenestrated capillaries which are separated from the epithelial cells by stromal tissue (which contains fibroblasts and immune cells – not shown). The epithelium has a well-defined apical brush border and basolateral interdigitations that greatly enhance the plasma membrane epithelial cell. The paracellular pathway is occluded by apically located tight junctions (TJ) although there may be still some paracellular movement of fluid (see text). The epithelium is well endowed with mitochondria indicative of high metabolic activity. BM = basement membrane.

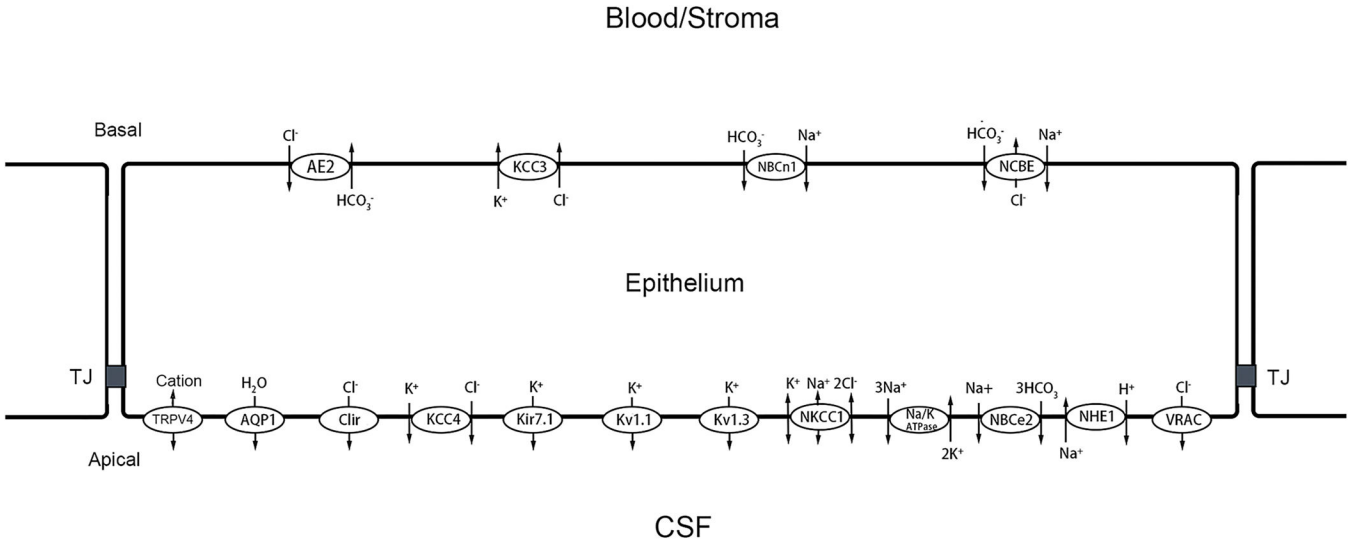

Fig. 2.

Polarized distribution of ion transporters at the choroid plexus epithelium. Multiple ion transporters and channels as well as the water channel, aquaporin 1 (AQP1), have a polarized distribution between the apical and basolateral membranes of the choroid plexus epithelium (reviewed in (Praetorius and Damkier, 2017), for apical TRPV4 see (Liedtke et al., 2000; Preston et al., 2018)). AE2, anion exchanger 2; KCC3, potassium chloride cotransporter 3; NBCn1, sodium bicarbonate cotransporter n1; NCBE, sodium chloride bicarbonate exchanger; $Cl_{ir,}$ inwardly rectifying chloride channel; KCC4, potassium chloride cotransporter 4; $K_{ir}7.1_{,}$ inwardly rectifying potassium channel 7.1; $K_v1.1_{,}$ voltage dependent potassium channel 1.1; $K_v1.1_{,}$ voltage dependent potassium channel 1.3; NKCC1, sodium potassium chloride cotransporter 1; Na/K ATPase; sodium potassium ATPase; NBCe2, sodium bicarbonate cotransporter e2; NHE1, sodium hydrogen exchanger 1; VRAC, volume regulated anion channel; TRPV4; transient receptor potential vanilloid 4, a non-selective cation channel. TJ, tight junction.

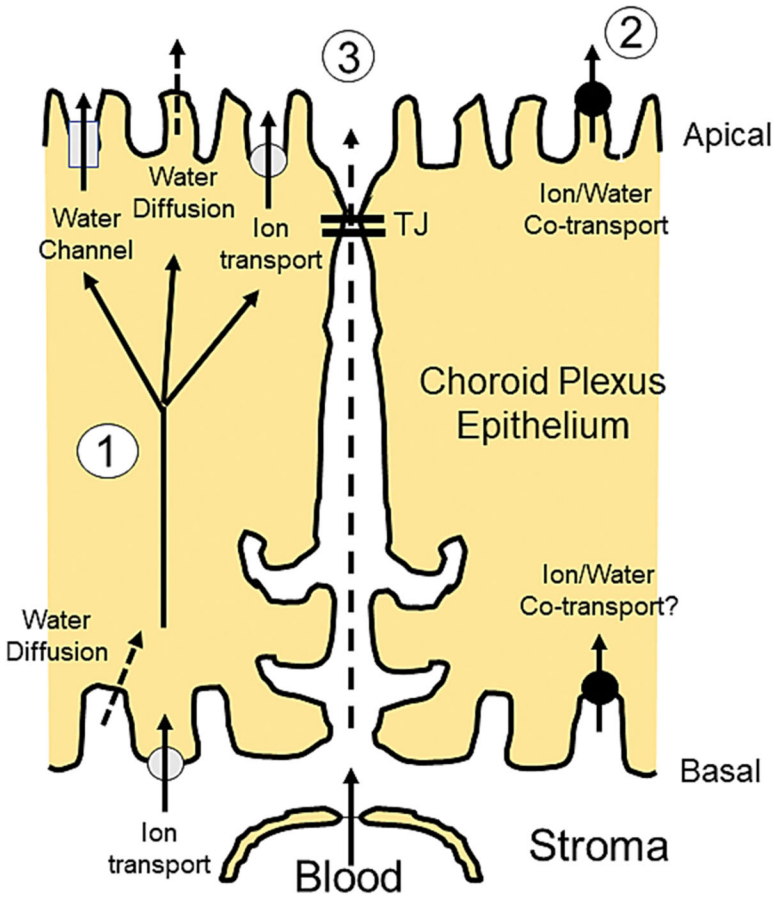

Fig. 3.

Proposed mechanisms of fluid secretion at the choroid plexus. The capillaries of the choroid plexus are fenestrated facilitating the movement of fluid from plasma to the choroid plexus stroma. Three models have been proposed (alone or in combination) for moving fluid across the choroid plexus epithelium. The polarized distribution of ion transporters may generate osmotic gradients that drive water movement (by diffusion or, at the apical membrane, via the water channel aquaporin 1) across the basolateral and apical membranes (1). The overall osmotic gradient between CSF and plasma is small and probably insufficient to account for CSF secretion (Razzaghi Khamesi et al., 2023). However, there may be very localized osmotic gradients in the basolateral interdigitations and between the apical microvilli. An alternative action of some of the ion transporters has been proposed (e.g., the apically distributed NKCC1) whereby they transport water as well as ions driving CSF secretion (2). Whether there are basolateral transporters with a similar function is unknown. The choroid plexus epithelial cells are linked by tight junctions (TJs) formed by transmembrane proteins occluding the paracellular space. Certain types of claudins that form TJs, can form ion and water permeable pores that may allow paracellular movement of fluid (3).

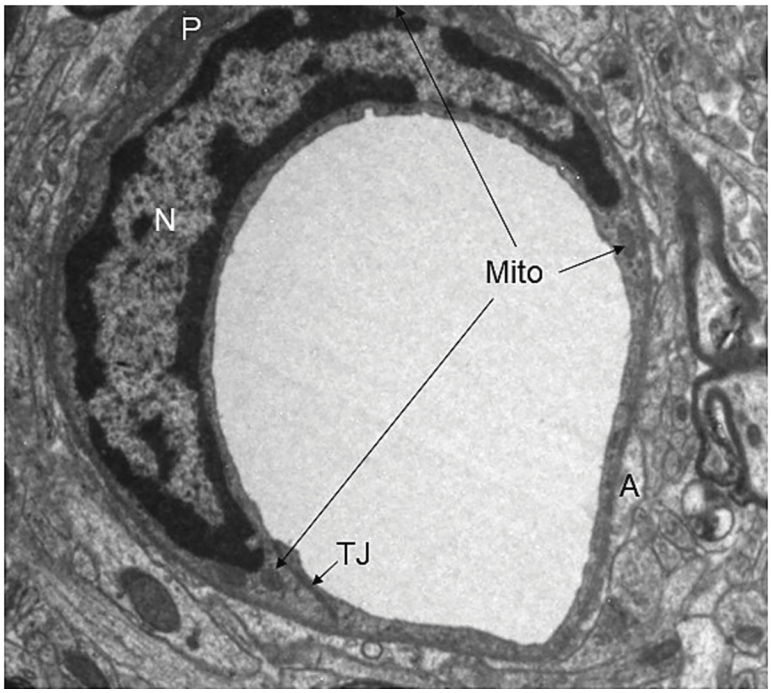

Fig. 4.

Rat cerebral capillary. Capillary endothelial cells are linked by tight junctions (TJ) have many mitochondria (mito) compared to systemic capillaries and a relative paucity of vesicles. They are partially covered by pericytes (P) and surrounded by astrocyte endfeet (A), important components of the neurovascular unit. N = endothelial nucleus.

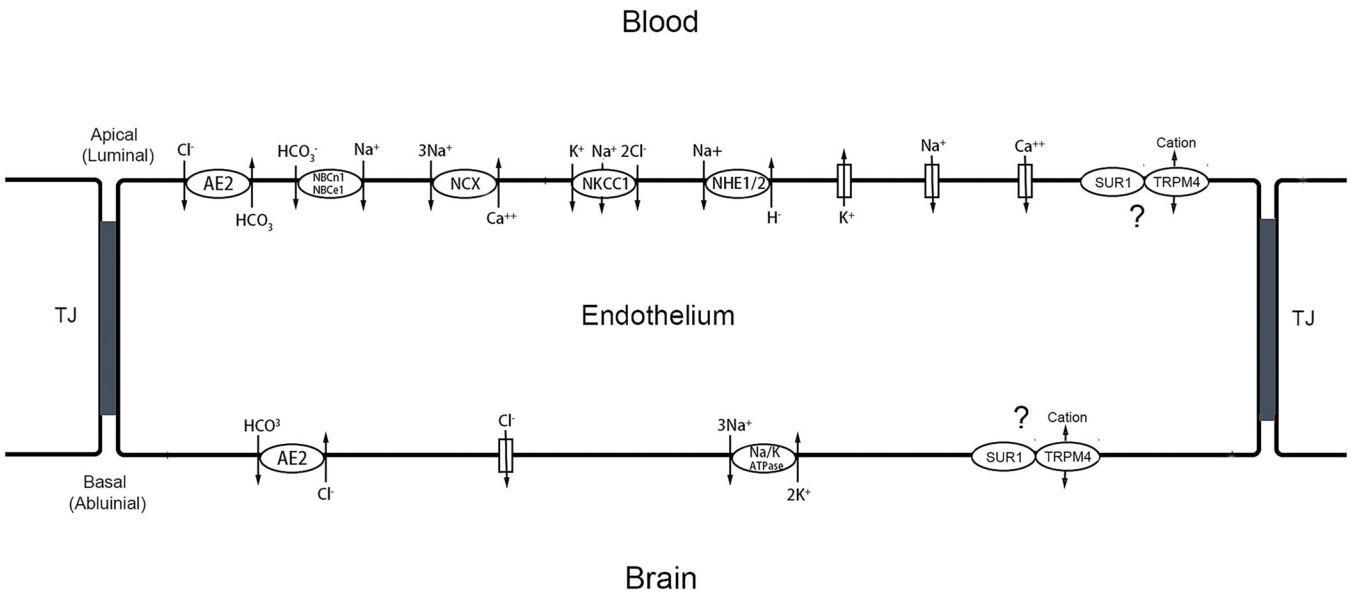

Fig. 5.

Polarized distribution of ion transporters at the cerebral endothelium. Transcriptomic studies have provided important information on which ion transporters and ion channels are expressed at the cerebral endothelium at the mRNA level (e.g., (Vanlandewijck et al., 2018)). However, less is known about the polarized distribution between the apical (luminal) and basal hampers the use of conventional light microscopy. Evidence here is predominantly from (O'Donnell, 2014). Recently, upregulation of a non-selective cation channel sulfonylurea receptor-1 (SUR1)-transient receptor potential cation channel subfamily M member 4 (TRPM4) has been reported after stroke (Simard et al., 2006; Woo et al., 2020). That channel has an important role in brain edema formation (Jha et al., 2021) but whether it has a polarized distribution at the cerebral endothelium is uncertain. AE2, anion exchanger 2; NBCn1, sodium bicarbonate cotransporter n1; NBCe1, sodium bicarbonate cotransporter e1; NCX, sodium calcium exchanger; NKCC1, sodium potassium chloride cotransporter 1; Na/K ATPase; sodium potassium ATPase; NBCe2, sodium bicarbonate cotransporter e2; NHE1/2, sodium hydrogen exchanger 1/2; TJ, tight junction.

Table 1

Different types of evidence for and against the choroid plexus being the major site of CSF secretion in mammals*.

For	Against
In vitro	
CP epithelial cultures can secrete fluid (Hakvoort et al., 1998a; Hakvoort et al., 1998b; Hulme et al., 2022)	
Fluid secretion by CP organoids (Pellegrini et al., 2020)	
In situ (animal)	
Perfused CP secretes fluid (Chen et al., 2009; Miner and Reed, 1972)	
In vivo (animal)	
Lateral ventricle CP extirpation reduces CSF secretion and Na^+ transport (Milhorat et al., 1976; Milhorat et al., 1971)	Cannulated aqueduct with no flow (Oresković and Klarica, 2010; Oreskovic et al., 2017)
Higher rate of blood-CSF vs BBB Na^+ and Cl^- transport (Spector et al., 2015a)	Block of CSF flow without ventriculomegaly and increased CSF pressure (Oresković and Klarica, 2010; Oreskovic et al., 2017)
Reduced CSF secretion with inhibitors of CP ion transporters (e.g., (Javaheri and Wagner, 1993; Steffensen et al., 2018))	Altered CP ion transporter expression can clear CSF (Sadegh et al., 2023; Xu et al., 2021)
Genetic deletion of CP ion transporters and Aqp1 reduce ventricle size/CSF secretion (Jacobs et al., 2008; Kao et al., 2011; Oshio et al., 2005)	
Induced or genetic block in 'CSF flow pathway' causes hydrocephalus (Lin et al., 2013; McAllister et al., 1991; Williams et al., 2014)	
Choroidal venous vs. arterial hematocrit (Welch, 1963)	
In vivo (human)	
Net caudal (anterograde) CSF flow at aqueduct on MRI (Eisma et al., 2023; Liu et al., 2022)	Net retrograde CSF flow at aqueduct on MRI in some neurological conditions (Eide et al., 2021; Kim et al., 1999)
CP cauterization + ETV for treating hydrocephalus (Stone and Warf, 2014; Warf, 2005; Warf et al., 2012)	Net retrograde CSF flow at aqueduct in children under 2 years-old (Bateman and Brown, 2012) (anterograde at older ages)
CP papillomas increase CSF secretion (Fujimura et al., 2004; Milhorat et al., 1976)	Cases where aqueduct stenosis does not cause hydrocephalus (Oresković and Klarica, 2010; Oreskovic et al., 2017)
Carbonic anhydrase inhibition of CSF secretion (Carrion et al., 2001)	Cases where CP cauterization does not reduce hydrocephalus (Oresković and Klarica, 2010; Oreskovic et al., 2017)
Block in 'CSF flow pathway' inducing hydrocephalus (Cinalli et al., 2011)	

CP, choroid plexus; ETV, endoscopic third ventriculostomy; MRI, magnetic resonance imaging.

*Some evidence is 'indirect'; e.g., measurements of flow or induction of ventriculomegaly does not specifically localize the source of CSF secretion (i.e., CP vs. transependymal). The use of transport inhibitors and global KO mice raise the possibility of 'non-CP' effects, while many of the surgical manipulations may alter brain and CP physiology.